

# A CYPRESS (*CUPRESSUS ARIZONICA*, CUPRESSACEAE) IN JEFF DAVIS COUNTY, TEXAS?

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## ABSTRACT

We investigated a recent report of a disjunct individual of *Cupressus arizonica* (Cupressaceae) collected in the Davis Mountains of Jeff Davis County, Texas. Chloroplast DNA sequences were obtained for the novel disjunct specimen and putative related taxa. Phylogenetic analysis suggests that the Davis Mountains *C. arizonica* specimen consists of vegetative tissue from *Juniperus deppeana*, a common species of the Davis Mountains. Based on this evidence, the present known distribution of *C. arizonica* in Texas remains restricted to the Chisos Mountains of southern Brewster County.

## RESUMEN

Hemos investigado un reciente registro de un individuo disyunto de *Cupressus arizonica* (Cupressaceae) colectado en las montañas de Davis del condado de Jeff Davis, Texas. Se obtuvieron secuencias de ADN cloroplastidial del nuevo registro y los supuestos taxa relacionados. El análisis filogenético muestra que el espécimen de *C. arizonica* de las montañas de Davis consiste en tejidos vegetativos de *Juniperus deppeana*, una especie común de las montañas de Davis. En base a esta evidencia, la actual distribución de *C. arizonica* en Texas, permanece restringida a las montañas Chisos del condado de Brewster.

## INTRODUCTION

*Cupressus arizonica*, as broadly circumscribed (Wolf & Wagener 1948; Eckenwalder 1993) includes a number of variable, isolated populations in southwestern North America. The recent report of a single individual of *Cupressus arizonica* at Bridge Gap Spring in the Davis Mountains of Jeff Davis County, Texas (Karges & Zech 2001) suggests a more continuous distribution in suitable habitats from California, through the southwestern United States, into Trans-Pecos Texas, and south into Mexico. Previously, *C. arizonica* was known from northern Mexico (Standley 1920; Wolf and Wagener 1948; Little 1978 Correll & Johnston 1979; Rehfeldt 1997) southern Brewster County, Texas (Powell 1988; 1998), and isolated localities in Arizona, California, and New Mexico (Sudworth 1927; Vines 1960; Little 1971; Minnich & Everett 2001). The Davis Mountains specimen led Karges and Zech (2001) to postulate a greater Pleistocene range for *C. arizonica*, represented by an occurrence in this additional Madrean sky island habitat.

The specimen upon which this phytogeographic postulate is based (Karges & Hedges 2480, SRSC) may not represent *Cupressus arizonica*, however. This

sheet consists of several short (< 12 cm), separate, yellowed terminal branches, and three small (< 8 mm), detached woody scales. Another voucher of the Bridge Gap putative *Cupressus arizonica* (Karges s.n., SRSC) consists of a single branch with green leaves, but no visible reproductive structures. Common members of the Cupressaceae that occur in the Davis Mountains are *Juniperus deppeana* var. *deppeana* and *J. pinchottii* (Powell 1998). Also present in the Davis Mountains, *J. deppeana* var. *sperryi* is an extremely rare endemic known from three individuals (Adams 1973; 1993; Watson & Eckenwalder 1993; Powell 1998). The Bridge Gap specimens could represent one of the above taxa, as concluded by B.L. Turner in an annotation (17 Jan 2001) of *Karges and Hedges 2480* as *Juniperus deppeana*. The distal vegetative growth of *Cupressus arizonica* and the above taxa of *Juniperus* are nearly indistinguishable. This may reflect on the sister relationship between these genera (Brunsfeld et al. 1994; Gadek et al. 2000).

The putative occurrence of *Cupressus arizonica* in the Davis Mountains is of biogeographic importance, and we attempt to address the veracity of the recent report in Jeff Davis Co., Texas, through an alternative means. Cupressaceous taxa that are difficult to identify by conventional means have been accurately identified through chemical analysis (Gough & Welch 1978; Adams 1993; Hsiang & Huang 2000). In order to verify the determination of the two Davis Mountains putative *Cupressus arizonica* specimens, we have gathered and compared chloroplast DNA sequence data from these specimens and specimens of putative related taxa.

#### METHODS AND MATERIALS

The Davis Mountains specimens were compared to 4 individuals representing *Cupressus arizonica*, 2 other *Cupressus* species, 11 individuals of *Juniperus*, and 1 specimen each of the outgroups *Calocedrus*, *Chamaecyperis*, and *Thuja* (Table 1). This sampling was based on the generic relationships within Cupressaceae suggested by recent studies (Brunsfeld et al. 1994; Gadek et al. 2000). Specimens used for DNA sequencing were either collected during fieldwork in Mexico and the United States in 2001, previously vouchered herbarium specimens, or vouchered live plantings growing at Rancho Santa Ana Botanic Garden (RSABG).

For all specimens collected in 2001, DNA was extracted using 2X CTAB, followed by precipitation in cold isopropanol (Friar et al. 1996). One gram of terminal shoot tissue (including leaves) was used for these extractions. For specimens collected before 2001, a modified small prep for dried leaf tissue was used for extractions (Doyle & Doyle 1987). Amplification of cpDNA templates of the *trnL* intron and *trnL-trnF* intergenic spacer follows the methods outlined by Porter et al. (2000). Purified template amplifications were sequenced directly with four primers, *trnLc*, *trnLd*, *trnLe*, and *trnLf* (Taberlet et al. 1991), using "big

TABLE 1. Specimens used in molecular analysis.

Species	Location	Voucher
<i>Calocedrus decurrens</i> Torr.	CALIFORNIA, Los Angeles Co.:	RSABG 13148 (RSA)
<i>Chamaecyparis lawsoniana</i> (A. Murr.) Parl.	CALIFORNIA, Del Norte Co.:	RSABG 14672 (RSA)
<i>Cupressus arizonica</i> Greene	TEXAS, Brewster Co.:	Bartel 571 (SRSC)
	TEXAS, Brewster Co.:	Bartel 572 (SRSC)
	TEXAS, Brewster Co.:	Warnock 7138 (SRSC)
	CALIFORNIA, San Diego Co.:	RSABG 11746 (RSA)
cf <i>Cupressus arizonica</i> Greene	TEXAS, Jeff Davis Co.:	Karges and Hedges 2480 (SRSC)
	TEXAS, Jeff Davis Co.:	Karges s.n. (SRSC)
<i>Cupressus lindleyi</i> Klotzsch ex Endl.	MEXICO, D.F.:	Griffith 325 (RSA)
	MEXICO, Michoacan:	Griffith 287 (RSA)
	CALIFORNIA, Shasta Co.:	RSABG 11434 (RSA)
<i>Cupressus macnabiana</i> A.Murr.	CALIFORNIA, Shasta Co.:	RSABG 11434 (RSA)
<i>Juniperus coahuilensis</i> (Martínez) Gausson ex Adams	TEXAS, Brewster Co.:	Bartel 575 (SRSC)
	TEXAS, Brewster Co.:	Powell 5146 (SRSC)
<i>Juniperus deppeana</i> Steud.	MEXICO, Puebla:	Griffith 324 (RSA)
	TEXAS, Jeff Davis Co.:	Powell 5186 (SRSC)
	TEXAS, Jeff Davis Co.:	Kolle 1193 (SRSC)
<i>Juniperus flaccida</i> Schlecht.	TEXAS, Brewster Co.:	Bartel 574 (SRSC)
	MEXICO, Coahuila:	Griffith 253 (RSA)
<i>Juniperus jaliscana</i> Martínez	MEXICO, Jalisco:	Griffith 279 (RSA)
<i>Juniperus monticola</i> Martínez	MEXICO, Oaxaca:	Griffith 304 (RSA)
<i>Juniperus pinchottii</i> Sudw.	TEXAS, Jeff Davis Co.:	Keough 205 (RSA)
<i>Juniperus virginiana</i> L.	TEXAS, Bastrop Co.:	Griffith 352 (RSA)
<i>Thuja plicata</i> Donn ex D. Don	CALIFORNIA, Humboldt Co.:	RSABG 13452 (RSA)

dye" chemistry from Applied Biosystems Incorporated, according to the manufacturer's specifications. All sequences were gathered using an Applied Biosystems Incorporated 3100 automated DNA sequencer.

Chromatograms derived from sequencing were assembled into contigs, and edited using Sequencher v4.1 (Gene Codes Corporation, Inc.). Consensus sequences were manually aligned using Se-Al v2.0a72 (Rambaut 1996). The aligned DNA data matrix is available from the first author upon request. The phylogenetic relationships among these specimens were estimated using Fitch parsimony, in PAUP\* v4.0β8 (Swofford 1998). Estimations of confidence in the clades were obtained through bootstrap analysis (Felsenstein 1985) with 10,000 pseudoreplicates, and through jackknifing (Farris et al. 1996), also with 10,000 pseudoreplicates (63% deletion) as implemented in PAUP\*.

#### RESULTS AND DISCUSSION

The specimens of *Cupressus arizonica* form a well-supported (94% bootstrap, 80% jackknife) monophyletic group with three other *Cupressus* specimens, and

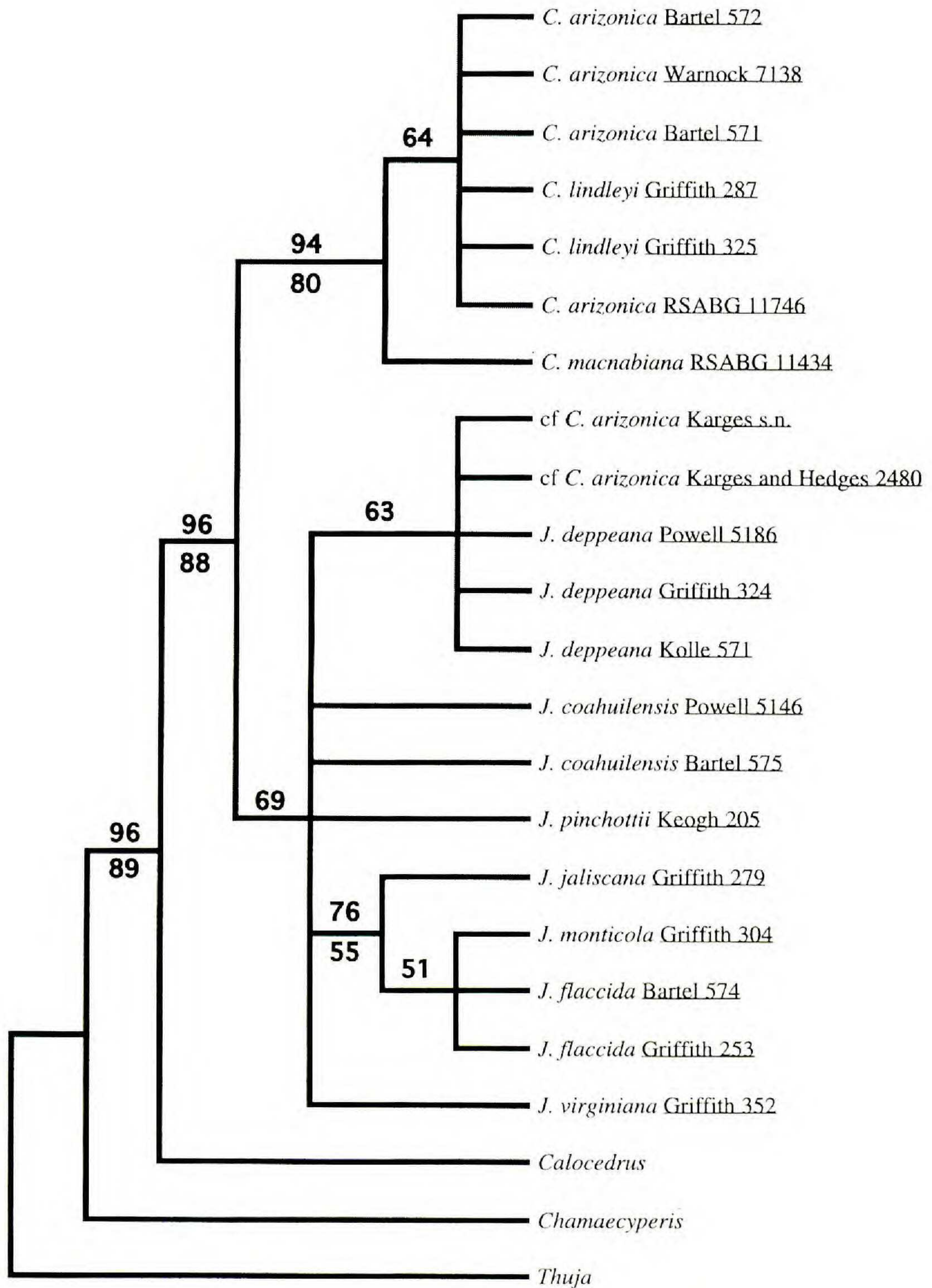


FIG. 1. A phylogenetic analysis of the two putative Davis Mountains *Cupressus arizonica* specimens (Karges and Hedges 2480 and Karges s.n.) and related taxa. Strict consensus tree of 258240 most parsimonious trees from the branch and bound analysis of the *trnL-F* region. With uninformative characters excluded, length = 47; CI = 0.7660; RI = 0.8854; rescaled consistency = 0.6782. Bootstrap percentages above 50% are indicated above the branches, and jackknife support above 50% is indicated below the branches. *C.* = *Cupressus*, *J.* = *Juniperus*.

this clade excludes the two Bridge Gap putative *C. arizonica* specimens (Fig. 1). The three specimens of *Juniperus deppeana* plus the two Bridge Gap specimens form a clade, though lacking strong support (63% bootstrap). *Juniperus deppeana* is a species present at Bridge Gap (Karges & Zech 2001). In context with the known flora of the Davis Mountains, chloroplast DNA data clearly suggest that the two Bridge Gap specimens are not *Cupressus arizonica*, and are most likely *Juniperus deppeana*.

The first author examined the Bridge Gap cypress closely in July 1999 and made the following observations: The specimen's trunk bark is divided into rough squarish plates near the base, characteristic of *Juniperus deppeana* var. *deppeana* (Powell 1988). At a height of about 0.5 m, the gray-brown bark is separated into longitudinal ridges, a key character for *Juniperus deppeana* var. *sperryi* (Adams 1973; Powell 1988). In contrast, the inner bark of *Cupressus arizonica* is often described as reddish (Wolf & Wagener 1948; Powell 1998), maturing often into thin fibrous strips on large trees (Correll & Johnston 1979; Bartel 1993), although the bark of *Cupressus arizonica* sensu lato can be quite variable. Martínez (1963) notes that *J. deppeana* var. *patoniana* forma *obscura* may have the lower bark checkered and the upper bark furrowed.

Although other characters may be of use, in most taxonomic keys the major feature diagnostic between *Juniperus* and *Cupressus* is the presence of fleshy, fused cones (*Juniperus*), versus woody, dehiscent cones (*Cupressus*) (Correll & Johnston 1979; Powell 1988; Bartel 1993, Watson & Eckenwalder 1993). Of the two specimens of the Bridge Gap putative *Cupressus*, one (Karges s.n.) is devoid of reproductive features and the other (Karges & Hedges 2480) has three separate orbicular-spathulate scales that are 5–7 mm long and 6–8 mm wide. These scales appear to have been sessile at the proximal end rather than peltate. *Cupressus arizonica* has 4–5 partially peltate proximal pentagonal scales approximately 13–15 mm long, 12–14 mm wide, and 2–3(–4) distal oblong, truncate scales 5–8 mm wide and 9–11 mm long, completely peltate and valvate (Table 2). Morphologically, the scales present on Karges & Hedges 2480 do not appear to be produced by *C. arizonica*.

One of the two putative *Cupressus arizonica* specimens (Karges s.n.) may be misdetermined because of a lack of reproductive characters, while the other (Karges & Hedges 2480) may have been determined as *C. arizonica* based on the three woody scales collected. Although the location of discovery of these three scales is faithfully recorded, we cannot be certain of their identity. Although unfortunate, this is not the first instance of a mixed collection being mistaken for something more significant (Thomson 1991). Given the above evidence, the present known distribution of *Cupressus arizonica* in Texas remains limited to the Chisos Mountains of southern Brewster County. This may change if a specimen exhibiting clear characters of *C. arizonica* is collected elsewhere in Texas.

TABLE 2. Specimens of *Cupressus arizonica* measured for morphological discussion.

Location	Voucher
California: San Diego Co.	Everett 33264 (RSA)
Mexico: Sonora.	Wolf 2564 (RSA)
Texas: Brewster Co.	Bartel 571 (SRSC)
Texas: Brewster Co.	Bartel 572 (SRSC)
Texas: Brewster Co.	Warnock 7138 (SRSC)

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